Assessment of myocardial necrosis is an important issue in patients with acute myocardial infarction (AMI) because size, transmurality and reperfusion are known predictors for outcome and survival (1–3). Left ventricular dysfunction following AMI can be irreversible when caused by necrosis with development of scar tissue, or reversible because of myocardial hibernation or myocardial stunning. Because the differentiation of reversible from irreversible myocardial injury influences therapy, there is growing interest in the prediction of myocardial viability.

Several methods have been used for noninvasive assessment of infarct size and definition of myocardial viability, including electrocardiogram (ECG) scores, cardiac enzyme measurements and echocardiographic and scintigraphic imaging techniques (4). The search for the optimal imaging strategy is ongoing, despite limitations including the inability to detect small infarctions, low sensitivity or specificity predicting myocardial viability and low spatial resolution.

In 1980, Goldman et al. (5) discussed the potential capabilities of cardiac magnetic resonance imaging (MRI). Given extensive hard- and software improvements over recent years, cardiac MRI is emerging as an alternative clinical tool for assessing myocardial infarction (MI). Acute MI results in increased myocardial signal on transversal relaxation time (T2)-weighted images. Attempts to use this property for accurate quantification of infarcted myocardium have largely been disappointing because of a systematic overestimation of the area of necrosis (6,7). Other studies investigated morphologic parameters (such as end-diastolic wall thickness) for the prediction of myocardial viability (8,9). These approaches, however, suffered from low specificity.

Several studies have investigated the ability of contrast-enhanced MRI to quantify infarct size. Furthermore, a number of promising MRI techniques have been proposed for the prediction of myocardial viability (10). However, some controversies about the most suitable contrast medium for imaging of MI persist. Whereas Kim et al. (11) demonstrated that the area of hyperenhancement 5 to 10 min after injection of extracellular contrast agents (such as gadolinium diethylene-triamino-penta-acetate [Gd-DTPA]) matches exactly the area of irreversibly damaged tissue on T1-weighted images, Saeed et al. (12) reported that these contrast media overestimate the area of necrosis. For accurate delineation of myocardial necrosis Saeed et al. (12) suggest the use of necrosis-specific, porphyrin-based contrast media. They further suggest that any area difference between the hyperenhanced regions demarcated by standard and necrosis-specific contrast agents could provide an estimation of potentially salvageable myocardium in the peri-infarction zone (10,12). To date there are no data available confirming or disproving this theory.

The aim of our study was to investigate whether the area of hyperenhancement using extracellular contrast agents is...
larger compared to the region demarcated by porphyrin-based contrast agents.

**MATERIALS AND METHODS**

All experimental protocols were performed in accordance with all state regulations governing animal experiments. The study was approved by the local “animal experiment” institutional review board. Myocardial infarction was induced in 15 rabbits (Belgian hare) of either gender (weight range 3.2 to 4.0 kg). The animals were premedicated with fentanyl (0.02 mg/kg, Fentanyl-Janssen, Janssen-Cilag, Neuss, Germany), midazolam (1 mg/kg, Midazolam, Hoffmann-La Roche, Grenzach-Wyhlen, Germany) and medetomidin intramuscularly (0.2 mg/kg, Domitor, Pfizer, Karlsruhe, Germany), anesthetized with propofol intravenously (1.5 mg/kg/min, Propofol Abbott, Abbott, Wiesbaden, Germany), intubated and mechanically ventilated. A left-sided thoracotomy was performed in the fourth intercostal space. A small incision was made in the pericardium and a permanent ligature was placed around a branch of the left coronary artery. The animals were divided into two groups: in 10 animals (group I) the magnetic resonance (MR) examination was performed 48 h following occlusion of the coronary artery for the evaluation of AMI. Animals of group II (n = 5) were imaged six weeks after occlusion of the coronary artery for evaluation of chronic myocardial infarction (CMI).

Both Gd-DTPA (Magnevist) and gadophrin-3 were prepared and supplied by Schering AG (Berlin, Germany). The necrosis-specific porphyrin-based contrast agent provides prolonged contrast between normal and pathologic tissue that last more than 24 h. The contrast between infarcted myocardium and surrounding tissue is best after a prolonged delay time, when gadophrin-3 leaves the intravascular space and accumulates in necrotic myocardium. Therefore, 50 μmol/kg of gadophrin-3 were injected into an ear vein 24 h before imaging. After collection of continuous gadophrin-3-enhanced short axis views, 100 μmol/kg of Magnevist was injected. Imaging was repeated following a delay time of 5 to 10 min.

All examinations were performed using a 1.5T scanner (Magnetom Symphony, Siemens Medical Systems, Erlangen, Germany) with a maximum gradient amplitude of 30 mT/m. A segmented k-space inversion recovery turbo fast low angle shot (IRturboFLASH) sequence designed to acquire a single high-resolution, strongly T1-weighted image was used for delayed enhancement imaging. Six to eight 3-mm slices without interslice gaps were used to cover the entire left ventricle. The field of view was reduced to a minimum of 176 mm. An acquisition matrix of 153 × 256 (5/8 rectangular field of view) resulted in a pixel size of 0.7 mm × 0.7 mm. A single-loop surface coil was used for data reception.

The IRturboFLASH sequence was optimized for imaging of small animals with a high heart rate (Fig. 1). In rabbits with an RR interval of about 400 ms, a trigger delay of 50 ms was used to push the acquisition window into late diastole. A nonselective inversion pulse was applied following the trigger delay. Data acquisition begins following an inversion time (TI) period which is defined as the time from the inversion pulse to the center of the acquisition window. Inversion time was optimized (typically between 180 and 240 ms) for each examination to null the signal of normal myocardium. The number of gradient echoes per segment was reduced to nine, resulting in an acquisition time of about 80 ms per segment, fast enough to make it insensitive to diastolic cardiac motion. Using a minimum repetition time (TR_{min}) of 300 ms, the maximum TI was 260 ms. The total acquisition time of the sequence amounted to 17 heartbeats (9 × 17 = 153 k-space lines).

The effective repetition time (TR_{eff}), which influences image contrast, can be calculated as the mean of the RR intervals during the measurement. Insufficient detection of the R-wave of the ECG results in varying repetition time values during the measurement and decreases image quality. Therefore, an active ECG system (Siemens Medical Systems, Erlangen, Germany) with an ECG amplifier next to the electrodes was used, as well as fiber optic data transmission. This system reduces ECG signal artifacts induced by the rapid switching of the gradients and ensured accurate triggering in all animals.

The animals were sacrificed following completion of the imaging protocol. The heart was excised and cut into short-axis slices (slice thickness 3 mm). The sections were immersed in 2% triphenyltetrazolium chloride (TTC) stain for 20 min at 37°C. The TTC-stained myocardial slices were photographed with a digital camera.

All images were transferred to an external workstation. The area of infarction was measured on TTC-stained as well as gadophrin-3 and gadophrin-3 plus Magnevist enhanced in-vivo images using commercially available software (Adobe Photoshop 5.0, Adobe, San Jose, California). The left ventricular endocardial and epicardial borders were

<table>
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<td>AMI</td>
<td>acute myocardial infarction</td>
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<td>CMI</td>
<td>chronic myocardial infarction</td>
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<td>ECG</td>
<td>electrocardiogram</td>
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<td>Gd-DTPA</td>
<td>gadolinium diethylene-triaminopenta-acetate</td>
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<tr>
<td>IRturboFLASH</td>
<td>inversion recovery turbo fast low angle shot</td>
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<td>MI</td>
<td>myocardial infarction</td>
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<td>MR</td>
<td>magnetic resonance</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<td>T1</td>
<td>longitudinal relaxation time</td>
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<td>TI</td>
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<td>effective repetition time</td>
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<td>TR_{min}</td>
<td>minimum repetition time</td>
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<td>TTC</td>
<td>triphenyltetrazolium chloride</td>
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**Abbreviations and Acronyms**

- AMI: acute myocardial infarction
- CMI: chronic myocardial infarction
- ECG: electrocardiogram
- Gd-DTPA: gadolinium diethylene-triaminopenta-acetate
- IRturboFLASH: inversion recovery turbo fast low angle shot
- MI: myocardial infarction
- MR: magnetic resonance
- MRI: magnetic resonance imaging
- T1: longitudinal relaxation time
- T2: transversal relaxation time
- TI: inversion time
- TR_{eff}: effective repetition time
- TR_{min}: minimum repetition time
- TTC: triphenyltetrazolium chloride
traced manually, and hyperenhancement was defined as signal intensity >3 SDs above the mean of normal myocardium. The MR images were analyzed in a random order by an observer blind to the TTC data.

The results of the measurements were compared by calculating systematic and random differences as well as the correlation coefficient. The agreement between the area of hyperenhancement on gadophrin-3 and gadophrin-3 plus Magnevist-enhanced images was illustrated in Bland and Altman graphs (13). The difference between the two measurements was calculated by subtracting the area of enhancement after additional injection of Magnevist from the area of enhancement following injection of gadophrin-3. The limits of agreement are provided as the mean ± SD.

The mean difference in size of hyperenhancement demarcated by gadophrin-3 and gadophrin-3 plus Magnevist-enhanced images was 1.8 ± 6.0 mm² (p < 0.05, paired t test); the calculated correlation coefficient was 0.99. Figures 2 and 3 demonstrate good correspondence between the area of infarction depicted on TTC staining and the hyperenhanced area seen on IRturboFLASH image 24 h after injection of gadophrin-3. The area of hyperenhancement does not increase following additional injection of a standard extracellular contrast agent. Figure 4 shows the excellent agreement of the two MR measurements plotted in a Bland and Altman graph.

For the comparison of in-vivo MR images and TTC staining, slices located at the margins of the infarctions were excluded in all animals because the evaluation of these slices may suffer from partial volume effects. The mean difference of infarction size defined by TTC staining and the area of hyperenhancement on MR scans was 6.3 ± 10.7 mm² for gadophrin-3 and 5.9 ± 13.1 mm² for gadophrin-3 plus Magnevist-enhanced images. Both MR measurements showed no statistically significant difference (p > 0.05, paired t test) compared to TTC staining.

Chronic MIs imaged six weeks following coronary artery occlusion showed no enhancement following gadophrin-3 injection, whereas application of Magnevist resulted in hyperenhancement of the infarction zone in all five animals of group II (Fig. 5). The spatial extent of hyperenhancement using Magnevist was virtually identical to the spatial extent of scar tissue defined by TTC staining (mean difference 5.8 ± 9.1 mm²; p > 0.05, paired t test).

**RESULTS**

Two animals with AMI (group I) died before imaging. Another two animals showed no area of hyperenhancement on gadophrin-3 or on gadophrin-3 plus Magnevist-enhanced in-vivo MR images. In these animals the experimental induction of MI had failed and MI could be excluded by TTC staining. The remaining six animals of group I showed hyperenhancement on two slices (n = 1), three slices (n = 1), four slices (n = 2) and seven slices (n = 2), resulting in a total of 27 hyperenhanced slices. The enhancement of the infarctions was homogenous in all animals.

The mean difference in size of hyperenhancement demarcated by gadophrin-3 and gadophrin-3 plus Magnevist was -1.8 ± 6.0 mm² (p > 0.05, paired t test); the calculated correlation coefficient was 0.99. Figures 2 and 3 demonstrate good correspondence between the area of infarction depicted on TTC staining and the hyperenhanced area seen on IRturboFLASH image 24 h after injection of gadophrin-3. The area of hyperenhancement does not increase following additional injection of a standard extracellular contrast agent. Figure 4 shows the excellent agreement of the two MR measurements plotted in a Bland and Altman graph.

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DISCUSSION

This study carries two important messages: 1) in acute nonreperfused MI the area of hyperenhancement on T1-weighted IRturboFLASH images following administration of an extracellular paramagnetic agent is not larger than the area of hyperenhancement seen on necrosis-specific gadophrin-3 enhanced images; and 2) six-week-old chronic MIs do not enhance with necrosis-specific gadophrin-3, but rather with an extracellular paramagnetic agent.

In contrast to earlier studies (12,14), there was no evidence for overestimating the size of AMI on the basis of the enhancement pattern of standard extracellular contrast agents. In the past various extracellular, intracellular, intravascular and necrosis-specific MR contrast agents have been evaluated for imaging AMI and CMI (10). To our knowledge, this is the first study comparing extracellular and necrosis-specific contrast agents in the same animal at the same time. Coincidence of the two measurements is of great importance, because the area of necrosis continues to grow as the peri-infarction zone of potentially salvageable myocardium becomes infarcted (12).

Figure 2. Acute transmural infarction (arrows). The area of hyperenhancement on gadophrin-3 (B) and gadophrin-3 plus Magnevist (C) enhanced images closely matches the infarction size defined by triphenyltetrazolium chloride staining (A).

Figure 3. An acute nontransmural infarction (arrows) can clearly be depicted on triphenyltetrazolium chloride staining (A), gadophrin-3 (B) and gadophrin-3 plus Magnevist (C) enhanced images. The area of hyperenhancement is virtually identical on both in-vivo magnetic resonance scans.
Contrast agents for the assessment of myocardial viability. In AMI, myocytes lose the ability to exclude standard extracellular contrast agents such as Gd-DTPA. Therefore the fractional distribution volume of the contrast agent increases. This increase can be detected as an area of hyperenhancement on T1-weighted images. Porphyrin-based necrosis-specific contrast agents, on the other hand, directly accumulate in infarcted myocardium (15). Although the exact mechanism of enhancement remains unknown, several studies demonstrated that the size of gadophrin-2

Figure 4. Agreement between area of hyperenhancement (mean value of the differences = closed line; ± 2 SD = dotted lines) from gadophrin-3 and gadophrin-3 plus Magnevist enhanced T1-weighted images depicted in a Bland and Altman graph. Average value of the two measurements is plotted along the x-axis; the difference (gadophrin-3 minus gadophrin-3 plus Magnevist) is plotted along the y-axis.

Figure 5. Diastolic (A) and systolic (B) cine magnetic resonance images show thinned myocardium with a lack of systolic wall thickening in an animal with chronic myocardial infarction. The inversion recovery turbo fast low angle shot sequence shows no enhancement 24 h after injection of gadophrin (C), whereas the infarcted myocardium (arrows) appears bright following the injection of gadolinium diethylene-triamino-penta-acetate (D).
(Schering AG) enhanced regions exactly matches the size of infarct defined by histologic staining (7,16). Gadophrin-3 (Schering AG) is a new porphyrin-based contrast agent. It differs from gadophrin-2 by the addition of a copper atom to the center of the phorphyrin system. Physicochemical and pharmacologic properties of both contrast agents are comparable, but the stability of the new contrast agent is considerably improved.

Saeed et al. (12) suggest the optimal MR contrast media for sizing of AMI to be characterized by long regional residence compared with image acquisition, and specific accumulation within necrotic myocardium, thereby providing high contrast between infarcted and normal myocardium. The first requirement has been fulfilled by hardware and software improvements of the MR systems. Magnetic resonance scanning has been vastly accelerated. Sequences are now capable of collecting high-resolution ECG-triggered images within the confines of a single comfortable breath-hold. Furthermore, new sequence designs have vastly improved the achievable contrast between infarcted and normal myocardium following injection of standard extracellular contrast agents. Thus, Simonetti et al. (17) reported that the contrast to noise level between normal and infarcted myocardium is about three times higher on segmented IRTurboFLASH sequences compared to standard T1-weighted spin echo or turbo spin echo sequences.

Some controversies concerning the choice of the most appropriate contrast agent for defining myocardial necrosis have persisted, however. Whereas Kim et al. (11) demonstrated that the area of hyperenhancement following injection of an extracellular contrast agent exactly matches irreversibly damaged myocardium, other groups (12,18) claimed an overestimation of infarction size by extracellular contrast agents. On the basis of this observation it has been suggested that the peri-infarction zone of injured but viable myocardium is about three times higher on segmented IRTurboFLASH sequences compared to standard T1-weighted spin echo or turbo spin echo sequences. Some controversies concerning the choice of the most appropriate contrast agent for defining myocardial necrosis have persisted, however. Whereas Kim et al. (11) demonstrated that the area of hyperenhancement following injection of an extracellular contrast agent exactly matches irreversibly damaged myocardium, other groups (12,18) claimed an overestimation of infarction size by extracellular contrast agents. On the basis of this observation it has been suggested that the peri-infarction zone of injured but viable myocardium is about three times higher on segmented IRTurboFLASH sequences compared to standard T1-weighted spin echo or turbo spin echo sequences.

Study limitations. This study has several limitations. First, only nonreperfused MIs were investigated. Reperfused infarctions may present with totally different patterns of enhancement. Second, as we aimed to image acute infarctions using two different contrast agents without a time delay, we had to compare gadophrin-3 and gadophrin-3 plus Magnevist-enhanced images. The absolute size of the infarction defined only by Magnevist enhancement could not be measured. However, overestimation of the infarct by Gd-DTPA could be excluded. Finally, comparison of in-vivo and postmortem images had to be performed using anatomical landmarks, because no epicardial markers were used in this infarct model.

CONCLUSIONS

Although some controversies persist, it can be concluded that imaging of delayed enhancement using extracellular contrast agents is a reliable tool for assessment of infarction size in AMI and CMI. In contrast to using Magnevist alone, the combination of gadophrin-3 and Magnevist can distinguish AMI and CMI because CMIs do not enhance with the necrosis-specific agent gadophrin-3.
REFERENCES